

Placental Transfer of Cadmium in Rats: Influence of Dose and Gestational Age

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Placental transfer rates of cadmium were investigated in rats in relation to dose (0.1, 0.4, and 1.6 mg Cd/kg) and the gestational age (12, 15, and 20 days) when rats were treated. Pregnant rats were injected intravenously with a single dose of $^{109}\text{CdCl}_2$ ($\sim 20 \mu\text{Ci/animal}$), and animals were sacrificed after 24 hr. ^{109}Cd concentrations were measured in the fetus, placenta, maternal liver, and blood.

Cadmium crossed the placenta at all doses and at all gestational ages tested. However, higher percentages of administered cadmium accumulated in the fetus with increasing dose and increasing gestational age. For example, after pregnant rats were injected with low, middle, and high doses of Cd on day 12 of gestation, fetuses accumulated 0.0001, 0.0028, and 0.0095% of the injected dose, respectively. Percentages of administered Cd detected in placental tissue did not change consistently with dose but Cd levels did increase with gestational age. Placental to maternal blood Cd concentration ratios increased with gestational age but not with dose. Maternal liver to fetal liver concentration ratios were 295, 137, and 27 for the low, middle and high doses, respectively, 24 hr after pregnant rats were treated on day 20 of gestation. These results are discussed in relation to placental damage, metallothionein inducibility, and fetotoxicity.

Introduction

Recently cadmium has been recognized to be a highly toxic environmental contaminant. Effects such as renal dysfunction and hypertension have been reported following long-term exposure (1,2). After injection of cadmium to experimental animals, fetotoxicity and teratogenicity were observed as well as necrotic changes in testicles and sensory ganglia. Reproductive effects and effects on

the offspring have also been found following long-term oral exposure of animals to cadmium (3). It is not known, however, whether the effects on the offspring seen in long-term exposures or those seen in acute exposures to cadmium are related to direct effects of cadmium transferred through the placenta.

Previous studies of Ferm et al. (4) demonstrated placental transfer of cadmium in hamsters after single intravenous administration of 0.50 or 0.85 mg Cd/kg on day 8 of gestation. On the other hand, Berlin and Ullberg (5), using an autoradiographic technique, did not detect placental cadmium transfer. Since it is well known that placental morphology and permeability characteristics change markedly during development, we attempted to elucidate the factors that regulate and

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influence placental transfer of heavy metals. Studies reported here were designed to give information regarding placental uptake and placental transfer of ^{109}Cd injected intravenously in the rat at various gestational times and after different doses.

Materials and Methods

Animals

Date-bred rats (Charles River, CD strain, 90–120 days old) were used. Fetal age was determined by designating the sperm positive date as day 0. The animals were housed singly in polycarbonate cages in an environment of controlled temperature ($22 \pm 1^\circ\text{C}$) and humidity ($50 \pm 10\%$), and they received tap water and Wayne Sterilizable Lab-Blox food *ad libitum*. Carrier-free ^{109}Cd as CdCl_2 in 0.5N HCl was obtained from New England Nuclear, Boston, Massachusetts. Injection solutions were prepared by dissolving CdCl_2 in saline (0.09%) and mixing with the $^{109}\text{CdCl}_2$ solution to obtain desired concentrations. Solutions were neutralized with 0.1N NaOH. Doses used were 0.1, 0.4, and 1.6 mg Cd/kg of body weight, and each rat was injected intravenously with 15–20 μCi of ^{109}Cd via the tail vein on day 12, 15, or 20 of gestation. Volumes were approximately 0.1 ml/100 g of body weight. Dose-response experiments were so designed that no embryotoxicity or teratogenicity was observed at the low dose whereas these effects were evident after pregnant animals received the high dose.

Tissue Analysis

Animals were anesthetized 24 hr after injection, and maternal whole blood was withdrawn by cardiac puncture. Part of the maternal livers, whole placentas, and whole fetuses were removed and placed in previously weighed plastic test tubes for gamma counting (Packard Instrument Co., Inc., Downers Grove, Illinois). Placentas and fetuses were washed immediately in saline, blotted with paper, and placed in gamma counting tubes. Fetal livers from animals treated on day 20 of gestation were removed, and livers from each litter were pooled. After removal of livers, individual fetuses were counted. Radioactivity measurements were performed in a Packard Autogamma scintillation counting system (6).

An attempt was made to assess placental function by using enzyme markers in animals treated with cadmium on day 20 of gestation. At 24 hr after treatment, placental cytosol (105,000g supernatant) was assayed for cystine aminopeptidase (7) and arylsulfatase A and B (8); L-cystine bisnitroanilide and 2-hydroxy-5-nitrophenyl sulfate, respectively, being used as substrates. Activity of serum heat-stable alkaline phosphatase was also measured by using *p*-nitrophenyl phosphate as the substrate (9).

Statistical Analysis

The significance of the results in relation to dose or gestational day was determined by Jonckheere's test (10). The values represent the mean \pm standard deviation unless otherwise mentioned.

Results

Cadmium levels in maternal liver and maternal whole blood 24 hr after injection expressed as percent of injected dose per gram of wet tissue are shown in Table 1. Liver values were consistently more than 100 times the blood values, which agrees with literature values (4). No significant differences in relation to dose or day of gestation were observed.

Percentages of injected dose of ^{109}Cd detected in placentas at various dose levels and gestational ages are illustrated in Figure 1. The Cd levels did not change consistently in relation to dose. However, significant ($p < 0.01$) gestational day differences were found for placental Cd levels for all three doses (Fig. 1). For example, the low dose treatment resulted in 0.10, 3.15, and 7.59% of the injected dose of ^{109}Cd in placentas after injection on day 12, 15, or 20 of gestation, respectively. The gestational day relationship (statistically significant at $p < 0.01$) was still evident in some cases when data were expressed as μg Cd/gram of placental tissue (Table 2). When data are calculated on a concentration basis, changes in tissue size do not influence the results.

Preliminary studies on placental function enzymes after Cd treatment on day 20 of gestation were not conclusive with no significant changes from control values. Enzymes tested were placental L-cystine aminopeptidase, arylsulfatases A and B and serum heat-stable alkaline phosphatase.

Percentages of injected doses of ^{109}Cd detected in fetuses 24 hr after treatment at different dose levels

Table 1. Cd in rat maternal liver and blood.^a

	Dose, mg/kg	Cd, %/g of injected dose ^b		
		Gestation day 12	Gestation day 15	Gestation day 20
Liver	0.1	(7) 2.9 ± 0.5	(5) 4.7 ± 1.2	(3) 5.1 ± 0.4
	0.4	(6) 4.7 ± 0.5	(3) 4.4 ± 0.6	(3) 4.7 ± 0.8^c
	1.6	(7) 3.0 ± 0.6	(3) 3.3 ± 1.2	(3) 3.4 ± 0.3^c
Blood	0.1	(6) 0.010 ± 0.015	(3) 0.017 ± 0.002	(4) 0.011 ± 0.002
	0.4	(7) 0.020 ± 0.009^c	(3) 0.022 ± 0.006	(3) 0.019 ± 0.008
	1.6	(8) 0.022 ± 0.008^c	(4) 0.017 ± 0.002	(3) 0.013 ± 0.004

^aRats sacrificed 24 hrs after Cd treatment at various dose levels.

^bValues in parentheses refer to numbers of animals.

^cSignificantly different from 0.1 mg/kg dose level, $p < 0.05$.

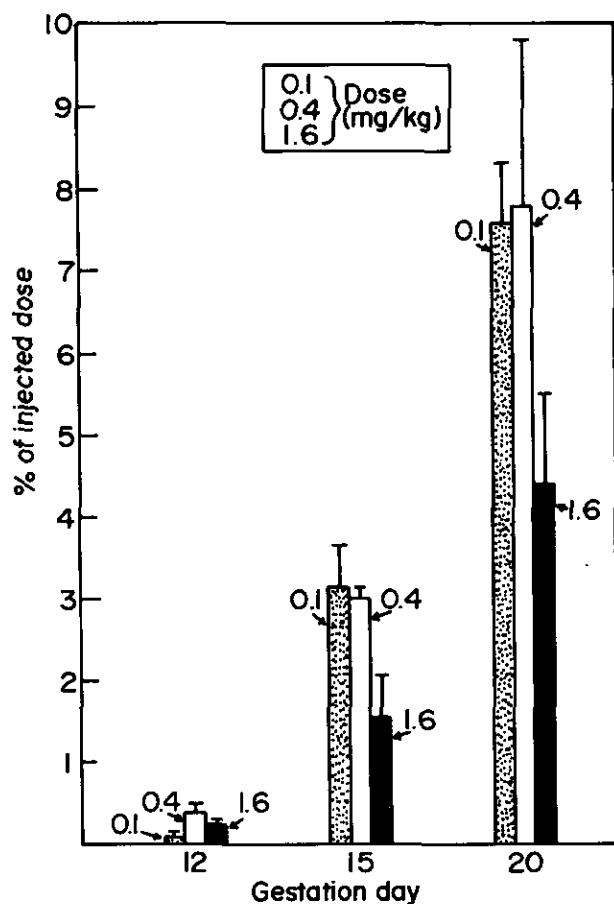


FIGURE 1. Percentage of injected ¹⁰⁹Cd detected in placentas for each dose and gestational day.

and gestational day periods are shown in Figure 2. The last bar graph represents the percentage of injected dose of ¹⁰⁹Cd in fetal rat livers treated on day 20 gestation. Fetal cadmium levels exhibited significant ($p < 0.01$) dose and gestational day relationships. Higher percentages of administered Cd were found in the fetuses with increasing dose and

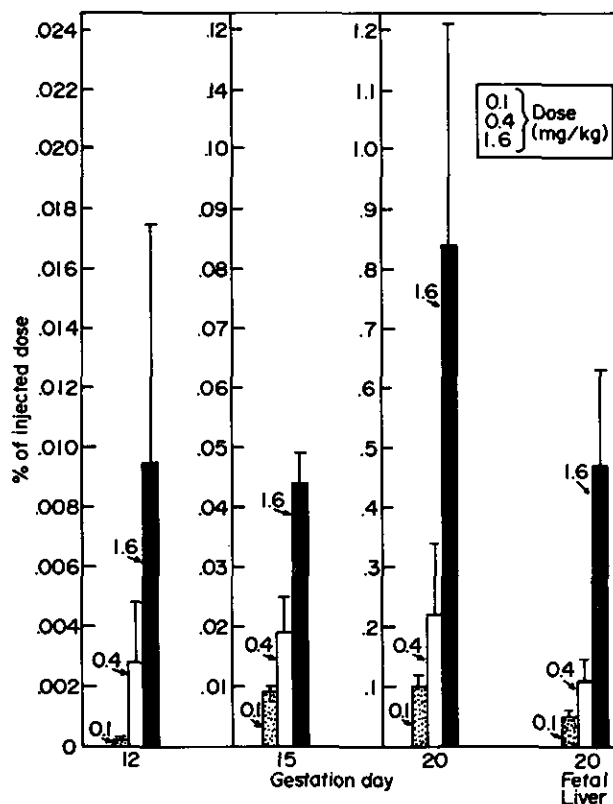


FIGURE 2. Percentage of injected ¹⁰⁹Cd detected in fetuses for each dose and gestational day.

Table 2. Cd in placentas and fetuses.^a

	Dose, mg/kg	Cd, % /g of injected dose ^b		
		Gestation day 12	Gestation day 15	Gestation day 20
Placentas ^e	0.1	0.063 ± 0.043 (1.6)	0.0115 ± 0.049 (5.8)	1.403 ± 0.293 (5.6)
	0.4	0.232 ± 0.032 ^c (1.6)	0.142 ± 0.024 ^d (4.7)	1.347 ± 0.127 (5.8)
	1.6	0.206 ± 0.057 ^c (1.3)	0.466 ± 0.147 ^d (3.8)	0.587 ± 0.167 (6.9)
Fetuses ^f	0.1	0.00012 ± 0.00004 (1.1)	0.0014 ± 0.0001 (6.8)	0.0019 ± 0.0003 (64.7)
	0.4	0.0022 ± 0.0023 ^d (1.1)	0.0027 ± 0.001 ^d (7.6)	0.0036 ± 0.0016 ^d (69.1)
	1.6	0.0217 ± 0.0111 ^d (0.9)	0.0096 ± 0.0026 ^d (4.9)	0.0152 ± 0.0058 ^d (50.8)

^aRats sacrificed 24 hr after Cd treatment at various dose levels.

^bNumbers in parentheses indicates average weight of placentas per litter or average fetal weight per litter. At least three pregnant animals were used to obtain each value.

^cSignificantly different from 0.1 mg/kg dose level, $p < 0.05$.

^dSignificantly different from 0.1 mg/kg dose level, $p < 0.01$.

^eThe dose-response test for placental data revealed significant dose-response relationships for the day 12 and day 15 groups and significant gestational day relationships at the 0.1 and 1.6 doses at least at $p < 0.05$.

^fThe dose-response test for fetal data revealed significant dose-response relationships for all gestational groups and significant gestational day differences at the 0.1 dose at least at $p < 0.01$.

increasing gestational age. For example, after pregnant rats were injected with low, middle, and high doses of Cd at day 12 of gestation, fetuses accumulated 0.0001, 0.003, and 0.009% of the injected dose, respectively (Fig. 2), representing a 90-fold increase in the percentage of cadmium detected in fetuses in the high dose compared to low dose. Fetal concentrations of Cd expressed as per cent of injected dose/gram are presented in Table 2. Significant gestational age relationships were observed when data were calculated as percentage of injected dose/gram (Table 2). For example, levels found in fetuses from animals treated with the low dose on day 20 of gestation were ten times those treated on day 12.

Attempts were made to assess possible differences in Cd uptake between fetuses dissected from right and left uterine horns or in individual fetuses from proximal or distal ends of the uterus. Although considerable variations were observed, no significant differences were found. The fetuses from the high dose treatment exhibited edema, hyperemia, and some of them were found dead. For example, high dose treatment on day 20 of gestation resulted in 14 dead fetuses out of 36. In other studies, when animals were treated on day 12 of gestation with 1.6 mg Cd/kg and sacrificed on day 21, teratogenic effects like cleft palate (2%), club foot (4%), and micrognathia (4%) were observed (51 fetuses were observed). No such effects were

detected in a corresponding number of animals receiving 0.1 or 0.4 mg Cd/kg.

In order to illustrate placental transfer rates of Cd the ratio of percentage of administered dose/gram tissue found in placenta to that detected in fetuses is presented in Table 3. Cadmium crossed the placenta at all gestational ages tested. The ratios clearly demonstrate some significant dose and gestational day relationships. In all groups there was more Cd in placentas than in fetuses. In general, placental:fetal ratios decreased with increasing dose and increasing gestational age on treatment. However, values were inconsistent for the high dose.

Table 4 shows the concentration of Cd in rat fetal liver following treatment on day 20 of gestation. Cd levels, as expected, increased in fetal liver with increasing dose levels. Percentages of fetal liver Cd of maternal liver levels exhibited a highly significant ($p < 0.01$) dose relationship with increasing percentages found in fetal livers with increasing dose. Fetal liver Cd levels account for approximately 50% of whole fetal Cd levels indicating that liver is the major Cd accumulation site in rat fetuses.

Maternal blood Cd is transported to the placenta where it may be bound and/or transferred to the fetus. Our data reveal that placenta to maternal blood concentration ratios increase with increasing gestational age for each dose tested (Table 5).

Table 3. Placental to fetal Cd concentration ratios.^a

Dose, mg/kg per day	Ratio placental: fetal Cd		
	Gestation day 12	Gestation day 15	Gestation day 20
0.1 ^c	618.00 ± 409.11	103.94 ± 29.28	78.877 ± 32.248
0.4	266.11 ± 232.92 ^b	55.92 ± 17.41	41.852 ± 16.786 ^b
1.6	11.23 ± 4.16 ^b	56.64 ± 7.56	42.69 ± 2.244 ^b

^aRatios are based on percentage of injected dose/g found in placentas and fetuses.

^bSignificantly different from 0.1 mg/kg dose level, $p < 0.01$.

^cThe dose-response test revealed significant dose-response relationships for the day 12 and day 15 groups at least at $p < 0.01$.

Table 4. Cd levels in rat fetal liver following treatment on day 20 of gestation.^a

Dose, mg/kg	Cd level		
	Fetal liver, μg/g	% of maternal liver	% of whole fetus
0.1 ^c	0.047 ± 0.011	0.22 ± 0.03	46.21 ± 6.89
0.4	0.092 ± 0.058 ^b	0.53 ± 0.26 ^b	49.69 ± 4.53
1.6	0.540 ± 0.200 ^b	2.93 ± 0.89 ^b	57.77 ± 4.58

^aRats sacrificed 24 hr after treatment at various dose levels. Three pregnant animals used to obtain each value.

^bSignificantly different from 0.1 mg/kg dose level at least at $p < 0.01$.

^cThe dose-response test revealed significant dose-response relationships for fetal liver cadmium concentrations and percentages of maternal liver at least at $p < 0.01$.

Table 5. Placental to maternal blood concentration ratios.^a

Dose, mg/kg	Ratio placental: maternal blood Cd		
	Gestation day 12	Gestation day 15	Gestation day 20
0.1 ^d	14.9 ± 3.0	37.9 ± 7.8 ^b	118.8 ± 47.7 ^{b,c}
0.4	12.3 ± 6.4	26.8 ± 4.5 ^b	58.7 ± 17.2 ^{b,c}
1.6	11.8 ± 7.9	25.9 ± 8.8 ^b	52.5 ± 36.3 ^{b,c}

^aRatio of percentage of injected dose/g of placentas to percentage of injected dose/g of maternal whole blood.

^bSignificantly different from the 12th day treatment, $p < 0.01$.

^cSignificantly different from 0.1 mg/kg dose level, $p < 0.05$.

^dThe dose-response test revealed significant gestational day differences for all doses at least at $p < 0.05$.

Discussion

Our data have demonstrated that the placental transfer rates of Cd increase with increasing gestational age. Maternal liver to fetal Cd percentages of injected dose range from about 500 in the low dose day 20 group to approximately 30 in the high dose day 20 group. In addition to the gestational age influence, dose effects were ascertained in the present studies. Previous studies by Ferm et al. (4) have shown that hamster placenta is permeable to Cd injected on day 8 of gestation. They suggested that the placental membranes might block the transfer of Cd as the placenta differentiates and matures.

This contrasts with our studies and might reflect species differences and age differences. The large concentration gradient between maternal and fetal tissues in our studies might explain why Berlin and Ullberg (5) could not detect fetal Cd in their autoradiographic studies. Previous studies (11) have also shown that Cd crosses the placenta in mouse strains whether they are sensitive or resistant to Cd teratogenicity after treatment on day 10 of gestation.

It is interesting to note that fetotoxicity and teratogenicity are observed after injection of the high dose at day 12 of gestation, when Cd levels in fetal and placental tissues are as low as 0.06 and

1.22 $\mu\text{g/g}$, respectively. These levels are similar to Cd levels after treatment at later gestational times when no fetotoxicity was observed, indicating greater fetal sensitivity to Cd toxicity at early developmental stages.

No correlation between placental function enzyme activities and placental transfer of Cd could be demonstrated. However, further studies are needed to clarify whether placental damage might influence the dose and gestational age relationships reported in this study.

In conclusion, our studies demonstrate that Cd injected intravenously passes the placenta and is detected in rat fetuses. The ratios of placental to fetal Cd concentrations decrease with increasing dose, and fetal percentages of injected dose/gram increase at later gestational ages as well as with dose.

A hypothetical explanation for these phenomena may be offered. It is known that an increase in Cd body burden initiates the biosynthesis of a Cd-binding protein-metallothionein (12). The ability of maternal tissues and placenta to bind cadmium and the inducibility rates of metal-binding protein in these tissues might be important factors in placental transfer. More cadmium might enter the

fetus when inducible cadmium-binding protein is saturated. Fetotoxicity may be related to maternal, placental, and fetal capacities to synthesize Cd-binding protein which theoretically acts as detoxication process for heavy metals. This hypothesis would also explain the observation that long-term low level exposure to Cd causes less teratogenicity and fetotoxicity than an equivalent single dose (3).

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